The developmental biology of Dishevelled: an enigmatic protein governing cell fate and cell polarity

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Summary

The Dishevelled protein regulates many developmental processes in animals ranging from *Hydra* to humans. Here, we discuss the various known signaling activities of this enigmatic protein and focus on the biological processes that Dishevelled controls. Through its many signaling activities, Dishevelled plays important roles in the embryo and the adult, ranging from cell-fate specification and cell polarity to social behavior. Dishevelled also has important roles in the governance of polarized cell divisions, in the directed migration of individual cells, and in cardiac development and neuronal structure and function.

Introduction

In its original definition, the word 'dishevelled' applied specifically to one's hair, its definition being 'without a hat' or, more commonly, 'un-coiffed'. Fittingly, the *dishevelled* gene was first identified and so named because in flies bearing this mutation the body and wing hairs fail to orient properly (Fahmy and Fahmy, 1959). (It may be of interest to some scientists, as it was to one of the authors, that dishevelled likely derives from the Spanish and French words for 'bald'.)

The *dishevelled* gene encodes a much-studied signal transduction protein that governs numerous biological processes. Nonetheless, our understanding of this protein has remained, well, dishevelled despite the identification of the first allele 50 years ago. It is clear that *dishevelled* encodes a protein that is an essential component of both the canonical WNT and the planar cell polarity (PCP) signaling cascades, and also signals via the WNT/Ca^{2+} pathway (Box 1, Fig. 1). The protein governs biological processes as distinct as cell polarity and cell fate specification, and even social behavior.

Much has been written about WNT signaling pathways, so this review will only briefly summarize our understanding of Dishevelled signaling activities and will instead concentrate on the biological processes that Dishevelled controls.

Dishevelled: an overview

The first alleles of the *dishevelled* (*dsh*) gene were identified in *Drosophila* mutants (Fahmy and Fahmy, 1959), and were characterized by disruptions in hair and bristle polarity. The locus attracted little attention until the 1980s, when it was discovered that this gene played a key role in segment polarity in the early embryo (Perrimon and Mahowald, 1987). Genetic experiments in early *Drosophila* embryos first placed Dsh in the canonical Wingless/WNT signaling pathway governing segment polarity (Couso et al., 1994; Noordermeer et al., 1994; Riggleman et al., 1990; Siegfried et al., 1994). Subsequent experiments demonstrated that *dishevelled* was also involved in the Frizzled-dependent signaling cascade governing PCP in the wing, legs and abdomen (Krasnow et al., 1995; Theisen et al., 1994). The cloning of the *Drosophila dsh* gene unveiled a novel protein (Klingensmith et al., 1994; Theisen et al., 1994), and additional experiments in the fly demonstrated that Dishevelled is positioned at the branchpoint between the canonical WNT and PCP signaling pathways (Axelrod et al., 1998; Boutros et al., 1998).

The first vertebrate homolog of *dishevelled*, murine *Dvl1*, was identified shortly thereafter, as was the *Xenopus* homolog *Xdsh*, two additional mouse genes (*Dvl2* and *Dvl3*) and three human DVL genes (Klingensmith et al., 1996; Pizzuti et al., 1996; Semenov and Snyder, 1997; Sokol et al., 1995; Sussman et al., 1994; Tsang et al., 1996). The vertebrate proteins are highly homologous to *Drosophila* Dishevelled, and experiments in *Xenopus* demonstrated that XDSH is an important part of the canonical WNT signaling cascade that controls early patterning in vertebrates (Dominguez et al., 1995; Sokol et al., 1995). In fact, interspecific experiments have demonstrated that Dishevelled function is highly conserved between flies, mice and frogs (Klingensmith et al., 1996; Rothbächer et al., 1995). More recently, vertebrate Dishevelled, like the fly counterpart, has been shown to also signal via a PCP cascade, controlling cell polarity during convergent extension cell movements that drive gastrulation (Heisenberg et al., 2000; Li et al., 1999b; Tada and Smith, 2000; Wallingford et al., 2000).

The structure of Dishevelled proteins

Dishevelled proteins are 500 to 600 amino acids in length and are modular. Each contains three highly conserved domains (Box 1, Fig. 2), and whereas the overall structure of Dishevelled has not been defined, structural descriptions of each of the three major domains have been reported (Box 1).

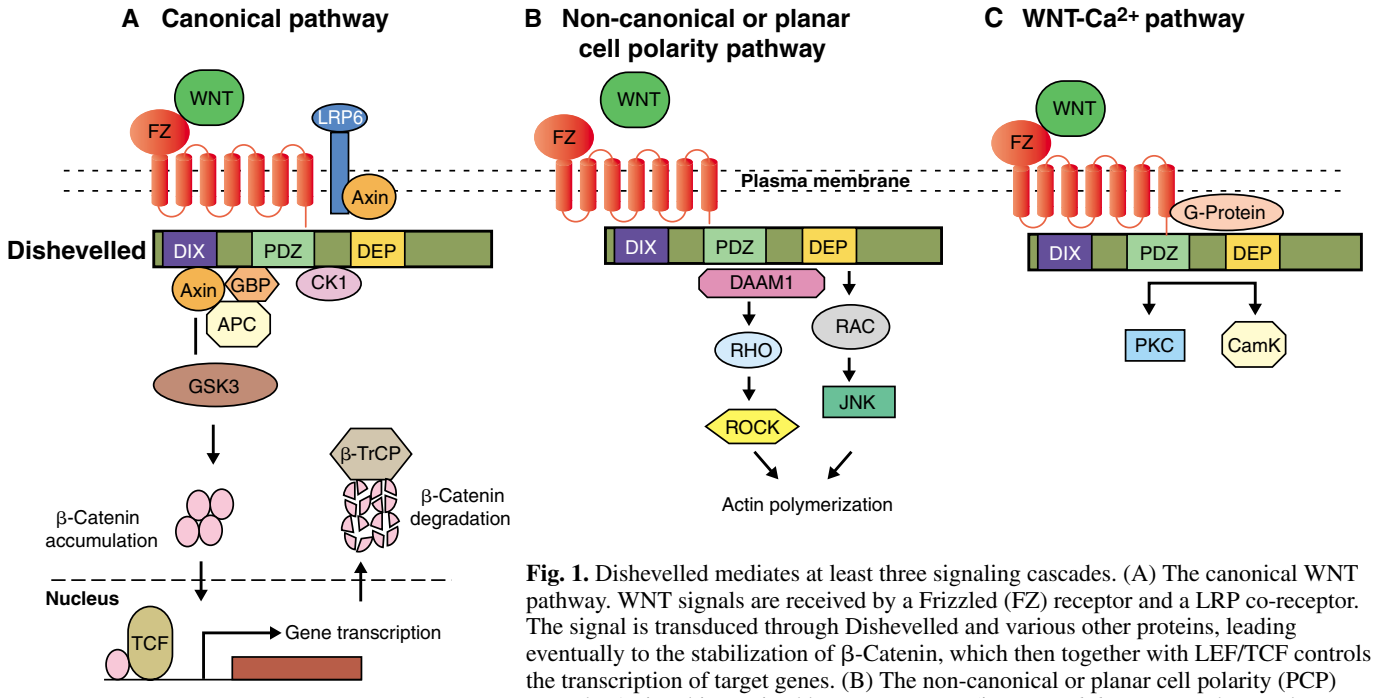


Fig. 1. Dishevelled mediates at least three signaling cascades. (A) The canonical WNT pathway. WNT signals are received by a Frizzled (FZ) receptor and a LRP co-receptor. The signal is transduced through Dishevelled and various other proteins, leading eventually to the stabilization of β-Catenin, which then together with LEF/TCF controls the transcription of target genes. (B) The non-canonical or planar cell polarity (PCP) cascade. A signal is received by a FZ receptor (in *Drosophila*, no WNT has yet been implicated in PCP signaling in vertebrates, although WNT11 and WNT5a are necessary), and transduced via Dishevelled to RAC and RHO, which then activate downstream targets to modulate the actin cytoskeleton. (C) The WNT/Ca^{2+} pathway. WNT signaling through the FZ receptors and Dishevelled and G-proteins leads to the release of intracellular calcium and signaling via Phospholipase C, CamK2 and PKC (Miller et al., 1999a; Sheldahl et al., 1999; Sheldahl et al., 2003). Image reproduced with permission from BioMedCentral (see Habas and Dawid, 2005). APC, Adenomatous Polyposis Coli; CKI, Casein kinase 1; CamK2, calcium/calmodulin-dependent kinase 2; GBP, GSK3 Binding Protein; GSK3, Glycogen synthase kinase 3; JNK, Jun kinase; LRP, Low-density lipoprotein receptor-related protein; PKC, Protein kinase C; TCF/LEF, Lymphoid Enhancer-Binding Factor/T-Cell Specific Transcription Factor; β-TrCP, Beta-Transducin Repeat Containing Protein; ROCK, RHO-associated coiled-coil forming kinase.

In addition to these commonly discussed domains of Dishevelled, several additional conserved regions deserve attention (Box 1, Fig. 2). For example, there is a basic region and scattered serine/threonine-rich stretches between the DIX and PDZ domains, and there is a proline-rich region with a SH3 protein-binding motif downstream of the PDZ (Penton et al., 2002; Rothbächer et al., 2000). A comparison of Dishevelled protein sequences from hydrazoan to human reveals the presence of several invariant residues downstream of the DEP domain, and the extreme C terminus is very highly conserved across species (Fig. 3). The significance of these conserved C-terminal residues for Dishevelled function remains unexplored.

Mechanisms of Dishevelled signaling

A fascinating aspect of Dishevelled is that this protein forms a branchpoint that links several widely deployed signaling pathways (Fig. 1). In this section, we discuss the proteins in the canonical WNT and PCP signaling pathways that function upstream and downstream of Dishevelled. As these signaling cascades have been extensively reviewed elsewhere, we will only attempt a thumbnail sketch of each pathway.

Upstream of Dishevelled: WNTs, receptors and co-receptors

With the sequencing of the human genome, nineteen WNT ligands have been identified (for reviews, see He et al., 2004; Logan and Nusse, 2004). The receptors for the WNT ligand were identified as members of the seven-pass Frizzled (FZ) gene family (see Huang and Klein, 2004). Members of the low-density lipoprotein-related receptor proteins (LRP), including *Drosophila* Arrow and vertebrate LRP5 and LRP6, function as co-receptors for WNTs (Pinson et al., 2000; Tamai et al., 2000; Wehrli et al., 2000).

As the LRP co-receptors only impinge on canonical signaling, it is possible that co-receptors may exist that channel WNT signals into the non-canonical arm and several proteins, including NRH1 [p75(NTR)-related transmembrane protein] (Chung et al., 2005; Sasai et al., 2004), protein tyrosine kinase 7 (PTK7) (Lu et al., 2004b), receptor tyrosine kinase-like orphan receptor 2 (ROR2) (Hikasa et al., 2002) and glypican (Topczewski et al., 2001), can modulate non-canonical WNT signaling. However, only ROR2 has been shown to bind to WNT (Hikasa et al., 2002), and as yet no evidence exists that these factors exist in a WNT/Frizzled complex to imply bona-fide co-receptor status for non-canonical signaling.

'Activation' of Dishevelled

Downstream of the WNT/FZ/LRP complex, signaling is transduced to Dishevelled, but it is hard to define the nature of 'activated' Dishevelled. Undoubtedly, Dishevelled becomes phosphorylated in response to WNT signaling, but the role of this phosphorylation remains unclear (Lee et al., 1999; Rothbächer et al., 2000; Willert et al., 1997; Yanagawa et al., 1995). Several kinases have been reported to phosphorylate Dishevelled, including Casein kinase 1ε, Casein kinase 2 and PAR1 (Cong et al., 2004a; Ossipova et al., 2005; Sun et al.,

2001; Willert et al., 1997). The mechanisms by which the signal is transduced to 'activate' Dishevelled remains mysterious and incompletely understood, although a recent report has demonstrated that FZ can directly bind to the PDZ domain of Dishevelled, albeit with weak affinity (Wong et al., 2003). The DEP domain of Dishevelled also appears to be involved in signal reception, as a deletion construct of mouse DVL1 containing only the DEP domain can inhibit the effects of overexpressed WNT, but not those of overexpressed wild-type DVL (Wong et al., 2000). Moreover, WNT signaling stimulates the DEP-mediated translocation of Dishevelled to the cell membrane (Axelrod et al., 1998; Boutros et al., 2000). In terms of downstream readouts, the mode of Dishevelled action is quite different depending upon which pathway is being discussed.

Canonical WNT signaling

At least two models have been advanced to explain the effects of Dishevelled on canonical WNT signaling (Fig. 1A) (for reviews, see Logan and Nusse, 2004; Seto and Bellen, 2004; Tolwinski and Wieschaus, 2004). In the first model, Dishevelled functions epistatically downstream of the Frizzled/LRP complex. To bolster this model, studies have revealed that the overexpression of Dishevelled can activate β-catenin signaling in *Drosophila* Arrow mutants (Wehrli et al., 2000), and that a constitutively active dFZ2-Arrow fusion cannot transduce signaling in a Dishevelled mutant background (Tolwinski et al., 2003). In this model, a likely mechanism by which Dishevelled functions is via the inhibition of Axin function (see below).

Dishevelled can bind Axin and inhibit its activity (Fagotto et al., 1999; Kishida et al., 1999; Li et al., 1999a; Smalley et al., 1999). Axin interacts with LRP5 (Mao et al., 2001) in a WNT-stimulated manner and is a potent negative regulator of WNT signaling (Zeng et al., 1997). It is possible that the interaction of Dishevelled with Axin is sufficient to inhibit the function of Axin, either through its sequestration or by the induction of its degradation. It is also noteworthy that both Axin and Dishevelled have been shown to cycle in and out of the nucleus, although the role of this nuclear shuttling remains unclear (Cliffe et al., 2003; Cong and Varmus, 2004; Habas and Dawid, 2005; Itoh et al., 2005). Furthermore, Frizzled overexpression can recruit Dishevelled to the membrane (Boutros et al., 2000; Rothbächer et al., 2000; Steitz et al., 1996), where Axin and Dishevelled have been found to be co-localized (Fagotto et al., 1999; Smalley et al., 1999). It is thus tempting to speculate that such interactions facilitate the binding of Dishevelled to Axin, thus activating canonical signaling.

In the second model, Dishevelled signals through a parallel pathway, whereby a FZ/Dishevelled complex functions independently from the FZ/LRP/Axin/Dishevelled complex. Support for this model comes from experiments in which the reduction of all Dishevelled isoforms from mammalian cultured cells did not impede signaling stimulated by an activated form of LRP (Li et al., 2002; Schweizer and Varmus, 2003). Another study has found that WNT-induced phosphorylation of Dishevelled can be achieved independently of LRPs (Gonzalez-Sancho et al., 2004)

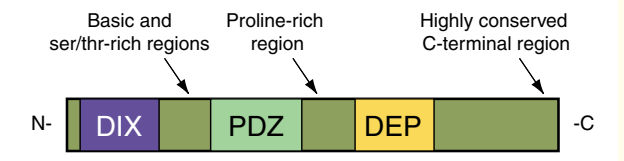
In the absence of WNT stimulation, β-catenin is targeted for degradation through the proteosomal pathway (Aberle et al., 1997; Liu et al., 2002). Whatever emerges as the mechanism by which Dishevelled functions, it appears safe to say that, upon WNT stimulation, Dishevelled acts to block the phosphorylation of β-catenin (Amit et al., 2002; Dominguez et al., 1995; van Noort et al., 2002). This in turn results in the cytoplasmic accumulation of β-catenin, which then traffics into the nucleus, where it complexes with members of the LEF/TCF family of transcription factors and induces the transcription of WNT-target genes (Bienz and Clevers, 2003; Liu et al., 2002; Yanagawa et al., 2002; Yost et al., 1996).

Planar cell polarity signaling

The 'non-canonical' WNT, or PCP, pathway signals downstream to the actin cytoskeleton and appears to be independent of transcription (Fig. 1B). A WNT signal, or another extracellular PCP signal, is received by a Frizzled receptor. Frizzled, and several other PCP effectors, including Strabismus, Diego and Prickle, then work together to govern the asymmetric accumulation of a complex of proteins at the plasma membrane that includes Dishevelled (for reviews, see Klein and Mlodzik, 2005; Veeman et al., 2003; Wallingford et al., 2002). Indeed, one recent study showed that Diego and Prickle competitively bind to Dishevelled to regulate its function in the PCP pathway (Jenny et al., 2005).

At the level of Dishevelled, two independent and parallel pathways lead downstream to the activation of the small GTPases RHO and RAC (Eaton et al., 1996; Fanto et al., 2000; Habas et al., 2003; Strutt et al., 1997; Tahinci and Symes, 2003). The first pathway signals to RHO, and occurs through the molecule DAAM1 (Dishevelled associated activator of morphogenesis 1) (Habas et al., 2001). This RHO pathway leads to the activation of the RHO-associated kinase ROCK,

Box 1. The conserved elements of Dishevelled protein



At the N terminus of Dishevelled is a DIX (Dishevelled/Axin) domain that is largely α-helical in structure (Capelluto et al., 2002). At its center lies a PDZ (PSD-95, DLG, ZO1) domain, which consists of six β-sheets that enfold two α-helices, which form a hydrophobic cleft that facilitates binding to other proteins (Cheyette et al., 2002; Wong et al., 2003). Slightly downstream from the PDZ domain lies a DEP (Dishevelled, EGL-10, Pleckstrin) domain, consisting of a bundle of three α-helices (Wong et al., 2000).

For 'canonical' WNT signaling, the DIX and PDZ domains of DSH are clearly used, and some contribution may also be made by the DEP domain (Axelrod et al., 1998; Boutros et al., 1998; Moriguchi et al., 1999; Penton et al., 2002; Rothbächer et al., 2000). For PCP signaling, the PDZ and DEP domains are used (Axelrod et al., 1998; Boutros et al., 1998; Heisenberg et al., 2000; Moriguchi et al., 1999; Tada and Smith, 2000; Wallingford et al., 2000). For WNT/Ca^{2+} signaling, the PDZ and DEP domains, but not the DIX domain, are essential (Sheldahl et al., 2003).

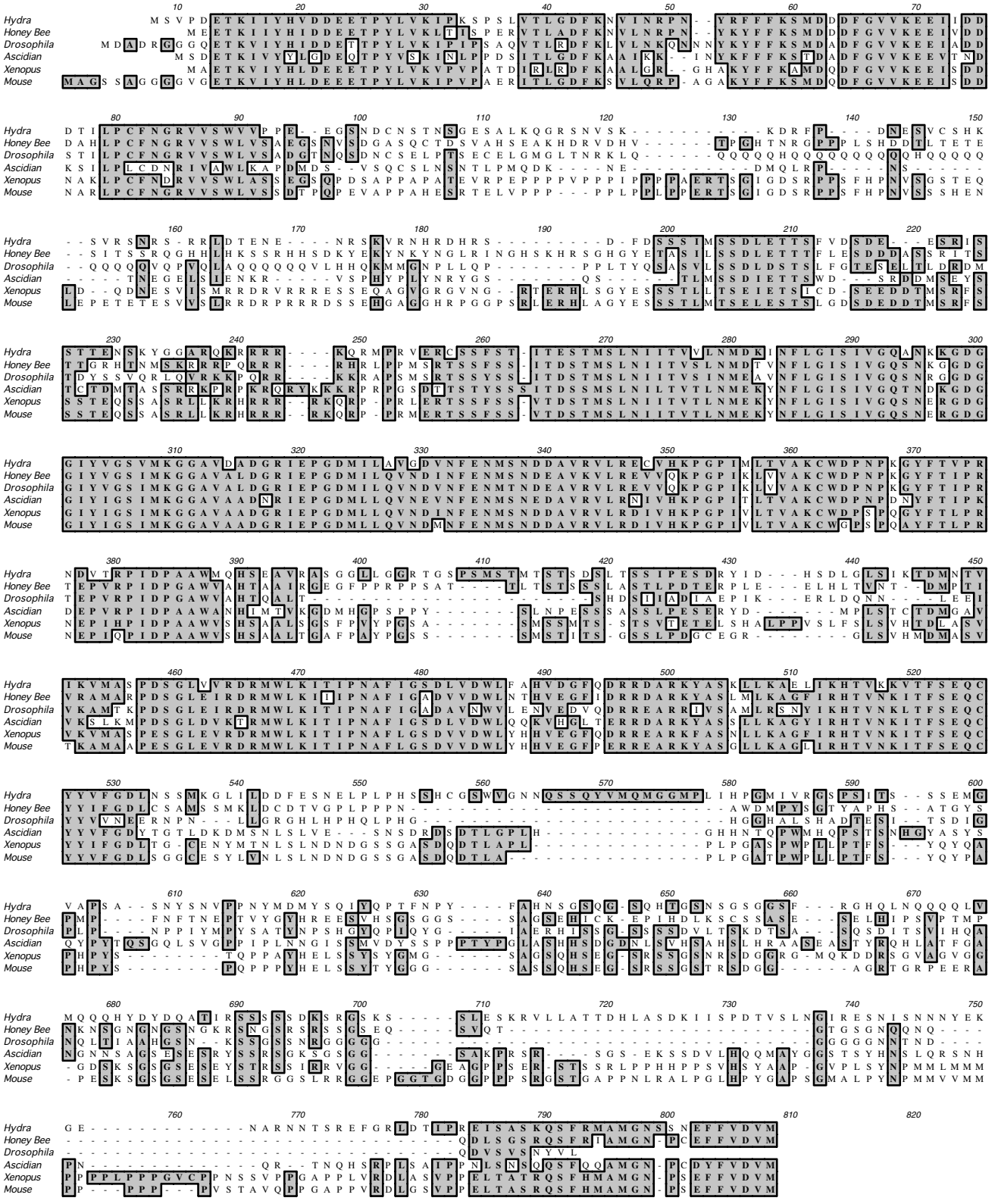


Fig. 2. Alignment of Dishevelled protein sequences from *Hydra*, honey bee, *Drosophila*, *Ciona*, *Xenopus* and mouse. The DIX domain covers approximately amino acid (aa) 15-90, the PDZ domain covers approximately aa 260-360, and the DEP domain covers approximately aa 460-530. Downstream of the DEP domain, an invariant proline, serine and two glycines can be found. The extreme C-terminal 24 amino acids are also very highly conserved from *Hydra* (and also in the planarian, not shown) to mouse. Curiously, although well conserved in the honey bee, this region is poorly conserved in published *Drosophila* DSH sequences, and a cursory BLAST search failed to find a similar sequence in the *Drosophila* genome.

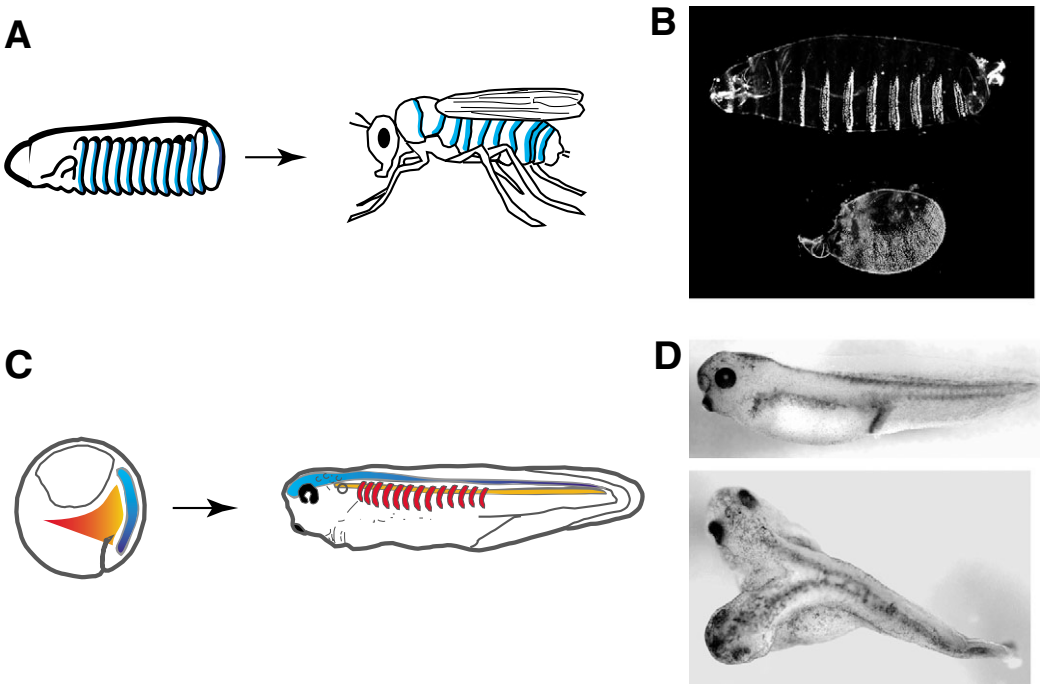


Fig. 3. Dishevelled-dependent canonical WNT signals govern embryo patterning. (A) Dishevelled is required to maintain segment polarity in *Drosophila*. A WNT signal (blue) is produced in the posterior of each segment and is received by Frizzled receptors on the anterior-most cell of the neighboring segment. This canonical WNT signal is transduced by Dishevelled. (B) A wild-type *Drosophila* embryo (top) and an embryo with disrupted canonical Wingless/WNT signaling (bottom). Images courtesy of K. Wharton (University of Texas, Southwestern Medical School). (C) Canonical WNT signals govern both dorsoventral patterning of the mesoderm (orange denotes dorsal; red, ventral) and anteroposterior patterning of the neuroectoderm (light blue denotes anterior; dark blue, posterior). (D) A normal *Xenopus* embryo (top) and an embryo with a duplicated axis resulting from ectopic ventral activation of canonical WNT signaling (bottom).

which mediates cytoskeletal re-organization (Kim and Han, 2005; Marlow et al., 2002; Veeman et al., 2003; Winter et al., 2001). A second pathway activates RAC, which in turns stimulates JNK activity (Boutros et al., 1998; Habas et al., 2003; Li et al., 1999b; Yamanaka et al., 2002).

It should be noted here that the true nature of Dishevelled function in the PCP cascade, as in the canonical pathway, remains mysterious. Several lines of evidence indicate that PCP signaling terminates with control of the actin cytoskeleton, and Dishevelled can activate the well-known actin regulators RHO and RAC (Habas et al., 2003; Tahinci and Symes, 2003). But it is also important to recognize that Dishevelled may speak to the actin cytoskeleton in a much more direct way. Dishevelled binds to DAAM1, a formin-homology protein (Habas et al., 2001), and formins are now recognized as being direct nucleators of linear actin cables (Sagot et al., 2002; Watanabe and Higashida, 2004). It seems likely that Dishevelled may employ multiple lines of communication to the actin cytoskeleton.

WNT/Ca^{2+} and beyond

Finally, other non-canonical pathways are now emerging. Foremost among them is the WNT/Ca^{2+} pathway, which may actually influence the function of both the canonical and PCP pathways (Fig. 1C) (Miller et al., 1999a; Sheldahl et al., 2003). In addition to the canonical and non-canonical WNT pathways, studies indicate that Dishevelled may also have 'supra-canonical' activities. For example, Dishevelled governs microtubule stability in neurons via Glycogen synthase kinase 3 (GSK3) and Axin, but not via other WNT pathway components (Krylova et al., 2000). Dishevelled also regulates cell migration in the *Drosophila* ovary in collaboration with WNT and Frizzled, but not with other components of the canonical or non-canonical pathways (Cohen et al., 2002).

Pathway specificity

Tremendous effort has gone into comprehending how Dishevelled channels a WNT signal into a distinct pathway. Two streams of thought have emerged, which are by no means mutually exclusive. The first involves the choice of effector molecules and the second, subcellular localization.

Binding partners

In over two decades of research, at least 29 Dishevelled-binding proteins have been identified (Table 1) (see also Wharton, 2003). How does one make sense of such a vast number of partners and how could they generate specificity? The WNT signal utilizes distinct domains of Dishevelled to branch signaling into the separate downstream pathways (Axelrod et al., 1998; Boutros et al., 1998). So, one convenient approach to synthesizing this information is to discuss the partners on the basis of the domains to which they bind.

The DIX domain of Dishevelled functions exclusively in canonical signaling and interacts with itself (homo-dimerization) and with Axin (Itoh et al., 2000; Julius et al., 2000; Kishida et al., 1999; Rothbächer et al., 2000; Smalley et al., 1999). When overexpressed, the DIX domain has been shown to act as a potent dominant-negative construct that

Table 1. Dishevelled-associated proteins*

Component	Dishevelled domain bound	Binding assay†	Role	Cellular localization	Pathway function	Reference
Dishevelled	DIX	Yeast two hybrid IP GST-pulldown	Agonist	Cytoplasmic Membrane Nuclear	GOF: canonical/PCP/Ca^{2+} LOF: canonical/PCP DN: canonical/PCP/ Ca^{2+}	Kishida et al., 1999; Rothbächer et al., 2000
PAK1	DIX	IP GST-pulldown	Agonist	Cytoplasmic Membrane	AchR clustering	Luo et al., 2002
Axin	DIX PDZ	Yeast two hybrid IP GST-pulldown	Antagonist	Cytoplasmic Membrane Nuclear	GOF: canonical LOF: canonical DN: canonical	Itoh et al., 2000; Julius et al., 2000; Kishida et al., 1999; Zeng et al., 1997
Frizzled	PDZ	Yeast two hybrid IP GST-pulldown	Agonist	Membrane	GOF: canonical/PCP/Ca^{2+} LOF: canonical/PCP DN: canonical/PCP	Cong et al., 2004; Wong et al., 2003
Casein Kinase 1	PDZ	IP GST-pulldown	Agonist	Cytoplasmic Nuclear	GOF: canonical LOF: canonical DN: canonical	Peters et al., 1999; Sakanaka et al., 1999
Casein Kinase 2	PDZ	Biochemical purification IP GST-pulldown	Agonist	Cytoplasmic Nuclear	GOF: canonical	Willert et al., 1997
GSK3-binding protein/ FRAT	PDZ	Yeast two hybrid IP	Agonist	Cytoplasmic	GOF: canonical LOF: canonical	Li et al., 1999; Yost et al., 1998
Frodo	PDZ	Yeast two hybrid IP	Agonist	Unknown	GOF: canonical LOF: canonical DN: canonical	Gloy et al., 2002
Dapper	PDZ	Yeast two hybrid IP‡ GST-pulldown	Antagonist	Cytoplasmic Nuclear?	GOF: canonical LOF: canonical/PCP DN: canonical	Cheyette et al., 2002
IDAX	PDZ	Yeast two hybrid IP GST-pulldown	Antagonist	Cytoplasmic	GOF: canonical LOF: canonical DN: canonical	Hino et al., 2001
Naked Cuticle	PDZ	Yeast two hybrid IP	Antagonist	Cytoplasmic	GOF: canonical/PCP LOF: canonical DN: canonical	Rousset et al., 2001; Wharton et al., 2001
Strabismus	PDZ	IP GST-pulldown	Agonist/ Antagonist	Membrane	GOF: PCP LOF: PCP DN: PCP	Bastock et al., 2003; Jessen et al., 2002; Park and Moon, 2002; Wolff and Rubin, 1998

Table 1 continued on next page.

effectively inhibits canonical WNT signaling downstream of the WNT/FZ/LRP complex in *Drosophila* embryos and *Xenopus* animal cap explants (Axelrod et al., 1998; Tamai et al., 2000). The PDZ domain of Dishevelled is the region most often found to interact with Dishevelled-binding proteins (note, most such studies actually use the PDZ, and also some flanking, sequnce). This central PDZ domain functions in both the canonical and non-canonical pathways. Through its interactions with the Dishevelled-binding partners, the PDZ domain has been proposed to function as the switch between the downstream pathways, depending on the binding partners that engage with it. Factors such as DAAM1, Strabismus, Prickle, Diego, and PAR1 can bind to the PDZ domain, and each functions in PCP signaling. Conversely, Casein kinase, GSK3, GBP/FRAT (GSK3 Binding Protein/Frequently Rearranged in T-Cell Lymphoma), Frodo, Dapper, Naked cuticle (NKD), Protein phosphatase 2, IDAX (Inhibitor of Dishevelled and Axin) and Daple each associate with the PDZ domain and function in canonical signaling.

The DEP domain functions in non-canonical signaling. This domain associates with, and mediates the activation of, the small GTPase RAC, which in turn leads to the activation of JUN kinase (Boutros et al., 1998; Habas et al., 2003; Li et al., 1999b). The requirement for the DEP domain in PCP is probably related to the fact that this region associates with RAC (Habas et al., 2003). The DEP domain also plays a central role in the cytoplasmic-to-membrane translocation of Dishevelled in response to WNT signaling or Frizzled overexpression (Axelrod et al., 1998; Krasnow et al., 1995; Pan et al., 2004; Rothbächer et al., 2000), but the mechanism by which this occurs and the protein factors required remain unknown.

Subcellular localization

Another mechanism by which Dishevelled specificity could be achieved is via its discrete subcellular localization. In *Drosophila*, some studies indicate that cytoplasmic Dishevelled is involved in canonical WNT signaling, whereas membrane-localized Dishevelled is important for PCP signaling (Axelrod et al., 1998). Conversely, the activation of

Table 1. Continued

Component	Dishevelled domain bound	Binding assay†	Role	Cellular localization	Pathway function	Reference
PAR1	PDZ	Biochemical purification IP	Agonist/ antagonist	Cytoplasmic?	PAR1BY GOF: PCP PAR1BY LOF: PCP PAR1A/BX GOF: canonical PAR1A/BX LOF: canonical	Ossipova et al., 2005; Sun et al., 2001
Diego/Diversin	PDZ	IP	Agonist/ Antagonist	Cytoplasmic	GOF: canonical + PCP LOF: PCP	Jenny et al., 2005
Protein Phosphatase 2C	PDZ	IP	Agonist	Cytoplasmic	GOF: canonical	Ratcliffe et al., 2000; Strovel et al., 2000; Yang et al., 2003
Notch	PDZ	Yeast two hybrid IP	ND	ND	ND	Axelrod et al., 1996
EPS8	PDZ	IP Yeast two hybrid	Unknown	Cytoplasmic?	Unknown	Inobe et al., 1999
Daple	PDZ	Yeast two hybrid IP Pulldown assay	Agonist	Unknown	GOF: canonical + PCP	Oshita et al., 2003
DAAM1	PDZ DEP	Yeast two hybrid IP‡ GST-pulldown	Agonist	Cytoplasmic Membrane	GOF: PCP LOF: PCP DN: PCP	R.H., unpublished
Prickle	DEP	IP GST-pulldown	Agonist	Cytoplasmic Membrane	GOF: PCP LOF: PCP DN: PCP	Jenny et al., 2005; Tree et al., 2002
MuSK	DEP	Yeast two hybrid IP‡ GST-pulldown	Agonist	Cytoplasmic Membrane	Acetylcholine receptor clustering	Luo et al., 2002
Disabled 2	DEP	IP	Antagonist	Membrane	GOF: canonical LOF: canonical	Hocevar et al., 2003
EphrinB1	Full length	IP GST-pulldown	Agonist?	Membrane	GOF: PCP?	Tanaka et al., 2003
PKCδ	Full length	IP	Agonist	Cytoplasmic Membrane	LOF: PCP DN: PCP	Kinoshita et al., 2003
NET1	Full length	IP	Antagonist?	Cytoplasmic	GOF: PCP	Miyakoshi et al., 2004
β-Arrestin	Full length	IP	Unknown	Cytoplasmic	GOF: receptor internalization	Chen et al., 2003

*A list of proteins that bind to Dishevelled, their region of interaction with Dishevelled and the binding assays used to detect interactions, the cellular localizations of the proteins and their function in which branch of the WNT pathway.

†Immunoprecipitation (IP) assays were performed using epitope-tagged protein, whereas GST-pulldown was performed using purified protein, unless stated otherwise.

‡Assay performed using both epitope-tagged and endogenous protein.

AchR, acetylcholine receptor; DN, dominant negative; GOF, gain of function; GST, Gluthathione-S-Transferase-fusion protein; IDAX, Inhibitor of Dishevelled and Axin; IP, immunoprecipitation; LOF, loss of function; ND, not done; PAR1A/BX, Partitioning Defective 1A/BX; PAR1BY, Partitioning Defective 1BY.

canonical WNT signaling is also associated with the membrane translocation of Dishevelled (Boutros et al., 2000; Yanagawa et al., 1995). Moreover, a recent study has shown that the localization of Dishevelled to apical versus basolateral membrane, rather than to membrane versus cytoplasm, influences pathway specificity (Wu et al., 2004). Indeed, this study showed that the pool of available Dishevelled is limiting, such that activation of one pathway sequesters Dishevelled in a particular location, leaving it unavailable to activate the other pathway (Wu et al., 2004). This finding may explain results that show that the activation of a non-canonical WNT pathway can downregulate canonical signaling, and vice-versa (Axelrod et al., 1998; Torres et al., 1996; Wu et al., 2004).

Clearly, binding partners and subcellular localization will be interdependent. In *Drosophila* wing epithelia, both pathway specificity and subcellular localization of Dishevelled can be directed by the cytoplasmic portions of distinct Frizzled receptors (Boutros et al., 2000; Wu et al., 2004). Frizzled receptors that are localized apically recruit Dishevelled to the apical plasma membrane and specifically transduce PCP signals, whereas canonical WNT signals use Frizzled receptors that are localized basolaterally and recruit Dishevelled to the basolateral membrane (Strigini and Cohen, 2000; Wu et al., 2004). It is likely that the mechanisms of Dishevelled pathway specificity will be cell-type and context dependent, and it will be interesting in the future to see whether a related mechanism of signal discrimination is at work in mesenchymal cells. For example, cells in *Xenopus* or zebrafish gastrula mesoderm have no defined apical or basolateral regions, yet still respond to both canonical and non-canonical WNT signals during gastrulation (see below).

In fact, the role of the subcellular localization of Dishevelled

in vertebrates remains poorly understood. The membrane translocation of Dishevelled is a commonly reported consequence of the activation of canonical WNT signaling in vertebrate cultured cells and in *Xenopus* animal caps (Boutros et al., 2000; Choi and Han, 2005; Steitz et al., 1996; Umbhauer et al., 2000; Yang-Snyder et al., 1996). By contrast, exposure of embryonic mouse kidney mesenchyme to WNT1 results in the accumulation of Dishevelled in and around the nucleus (Torres and Nelson, 2000). Additionally, the association of Dishevelled with punctate intracellular vesicles was found to be required for canonical WNT signaling in Chinese hamster ovary (CHO) culture cells (Capelluto et al., 2002) and in *Xenopus* animal caps (Choi and Han, 2005). However, in HEK293 cells (a human embryonic kidney cell line), such punctate localization of Dishevelled correlates with decreased canonical WNT signaling (Cong et al., 2004a). Finally, in the mesoderm of gastrulating *Xenopus* embryos, Dishevelled is localized to the plasma membrane in cells undergoing convergent extension, but not in other cells (Wallingford et al., 2000).

Three very recent studies have further highlighted the interplay between Dishevelled's binding partners and its subcellular localization during vertebrate convergent extension. One study revealed that the phosphorylation of Dishevelled by different isoforms of PAR1 in *Xenopus* resulted in the transduction of either canonical WNT or PCP signals (Ossipova et al., 2005). Notably, phosphorylation by the PCP-specific isoform of PAR1 was associated with the translocation of Dishevelled to the cell membrane. When such phosphorylation was blocked, Dishevelled failed to accumulate at the membrane and PCP signaling was disrupted (Ossipova et al., 2005). Consistent with this result, another study used engineered constructs to sequester Dishevelled at specific sites in the cell, so demonstrating that the membrane localization of Dishevelled is essential for PCP signaling (Park et al., 2005). By contrast, the particular subcellular location of Dishevelled did not influence the activation of canonical WNT signaling. Instead, constructs that caused Dishevelled to be sequestered to either the cell membrane or to the mitochondrial membrane were more active in canonical signaling than was an equivalent amount of wild-type Dishevelled (Park et al., 2005). The final study showed that inversin, a vertebrate ortholog of the *Drosophila* PCP effector Diego, activates PCP signaling and simultaneously downregulates canonical WNT signaling by targeting Dishevelled for proteosome-mediated degradation. Intriguingly, these authors show that membrane-targeted Dishevelled is protected from such degradation (Simons et al., 2005).

The biology of Dishevelled signaling

In this section, we focus on the biological processes that are governed by the many signaling capabilities of Dishevelled. We start with a summary of classical settings in which Dishevelled function has been studied, and proceed to more recently described functions.

Drosophila segment polarity

Dishevelled was first identified in *Drosophila* as a viable mutant that had obvious defects in the orientation of the bristles on the wing and thorax of the fly (Fahmy and Fahmy, 1959). Dishevelled was also identified in later genetic screens in which removal of the maternal/zygotic product phenocopied Wingless and Armadillo/β-Catenin mutants, indicating that Dishevelled has an early critical function in larval patterning in *Drosophila* (Fig. 3A,B) (Nusslein-Volhard and Wieschaus, 1980; Perrimon and Mahowald, 1987). This role and Dishevelled's placement in the Wingless/WNT pathway was demonstrated by additional studies in the fly embryo (Couso et al., 1994; Noordermeer et al., 1994; Riggleman et al., 1990; Siegfried et al., 1994) and wing (e.g. Heslip et al., 1997).

Vertebrate dorsoventral axis patterning

The *Xenopus* model system has been pivotal in uncovering the mechanism of WNT/Dishevelled signaling in early pattern formation during vertebrate embryogenesis (Fig. 3C,D) (Harland and Gerhart, 1997). An endogenous complex, which translocates to the future dorsalizing center of the early frog embryo, does indeed contain Dishevelled (Miller et al., 1999b). The translocation of Dishevelled and other factors likely restricts β-Catenin activation to the future dorsal region (Larabell et al., 1997). Recently, *Xwnt11* was shown to be expressed maternally and to translocate dorsally during cortical rotation, probably completing the picture of how the canonical WNT pathway specifies dorsal fates (Tao et al., 2005).

A word of caution must be inserted here. No complete loss-of-function study of *Xenopus* Dishevelled has been reported. Therefore, its requirement for early patterning remains a thorny issue. Indeed, mutant mice lacking both *dvl1* and *dvl2* have revealed a requirement for Dishevelled during neural fold closure and cardiac development, but not during early axial patterning (Hamblet et al., 2002; Lijam et al., 1997). Nonetheless, a Dishevelled-mediated system of early axis patterning appears to be very ancient and is implicated not only in *Xenopus*, but also in sea urchins and even hydrazoans (Hobmayer et al., 2000; Weitzel et al., 2004).

Drosophila wing hair polarity

The hairs and bristles that cover the body and wings of insects are highly polarized; all point posteriorly on the body and distally on the wing. This issue attracted attention as early as 1940 (Wigglesworth, 1940), and subsequently became a topic of much broader interest (for reviews, see Adler, 1992; Lawrence, 1973).

Early studies suggested that polarity information is provided by a supracellular gradient of spatial information (non-cell autonomous), and that a cell-autonomous mechanism also acts to maintain cell polarity (see Wigglesworth, 1959). Evidence that Dishevelled is a crucial player in this latter process came from the observation that disruptions of its function disturbed the establishment of planar polarity. The dsh^{1} allele, which harbors a mutation in the DEP domain, disrupts the polarity of wing hairs, abdominal bristles, and bracts on the legs (Theisen et al., 1994). Although mutation of the Frizzled receptor results in non-cell-autonomous defects in planar polarity (Vinson and Adler, 1987), Dishevelled affects planar polarity in a purely cell-autonomous manner (Klingensmith et al., 1994; Theisen et al., 1994).

In the case of wing hairs, planar polarity is established by the outgrowth of an actin-rich prehair exclusively from the distal vertex of each cell (Fig. 4A,B,E) (Wong and Adler, 1993). Directed actin polymerization at the distal vertex

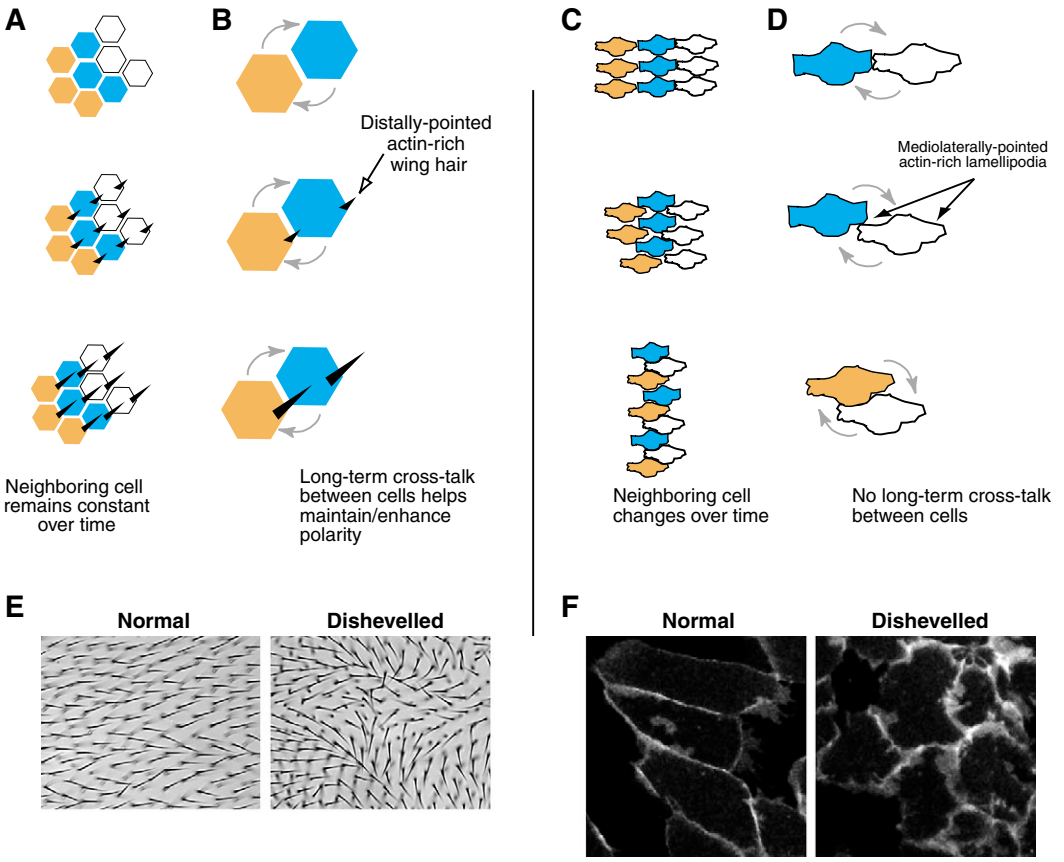


Fig. 4. Dishevelled-dependent PCP signaling governs cell polarity. (A) A schematic of planar polarity establishment in the *Drosophila* wing (a higher magnification view is shown in B). Dishevelled-dependent PCP signaling coordinates the orientation of distally pointing, actin-rich hairs in these cells. The arrangement of these cells remains constant, and long-term cross-talk between neighboring cells is essential for polarity establishment. (C) A schematic of planar polarity during convergent extension in *Xenopus* (a higher magnification view is shown in D). Convergent extension is driven by polarized cell interdigitation. By crawling between one another along a single axis, the population of cells is converted from being short and wide to long and narrow. Dishevelled-dependent PCP signaling is essential to the stabilization of lamellipodia specifically on the mediolateral faces of these cells. Unlike *Drosophila* wing cells, these cells are constantly changing neighbours. How the PCP signaling cascade has changed to accommodate this dynamic situation is a topic of great interest. (E) Wing hairs on a normal *Drosophila* embryo all point distally, but such polarity is perturbed in an embryo with disrupted Dishevelled signaling (images courtesy of Jeff Axelrod, Stanford University School of Medicine). (F) *Xenopus* mesoderm cells engaged in convergent extension are aligned and polarized, but this polarity is perturbed in an embryo with disrupted Dishevelled signaling.

governs hair outgrowth. In Dishevelled mutant flies, the abnormal positioning of prehairs presages the disrupted polarity of the hairs themselves (Wong and Adler, 1993). This fundamental finding suggested that Dishevelled controls actin polymerization. Importantly, Dishevelled protein accumulates asymmetrically at the site of prehair initiation (Axelrod, 2001).

The planar polarity of *Drosophila* wing cells has become a gold standard for studies of PCP signaling, perhaps in part because of the ‘simplicity’ of the system: the polarization of individual, monopolar cells that maintain a constant relationship with their neighbors (Fig. 4A,B). Indeed, much of what we know about the mechanisms of Dishevelled function and PCP signaling comes from studies in the fly wing. Continued investigations in this system will certainly help to elucidate the molecular basis of Dishevelled function.

Drosophila ommatidal polarity

Dishevelled is also required for planar polarity in the eye, where polarity is manifested by groups of cells, rather than by individual ones. To add another layer of complexity, each ommatidium is itself asymmetric. As such, polarity in the eye is manifested by the chirality or handedness of each ommatidial cluster, and also by the orientation of the overall cluster. Mutants have revealed that Dishevelled is necessary for both normal chirality and normal overall orientation (Theisen et al., 1994). Despite early suggestions to the contrary, several additional genetic studies have demonstrated that Dishevelled signals through the PCP cascade to control these aspects of ommatidial planar polarity (Boutros et al., 1998; Zheng et al., 1995).

Interestingly, the chirality of ommatidia depends upon a fate choice in the R3/R4 pair of photoreceptor cells, which in turn depends upon the modulation of Notch signaling downstream of a Dishevelled-mediated PCP signal (Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Strutt et al., 2002). In fact, there has been at least one report of a physical association between Dishevelled and Notch (Axelrod et al., 1996). This is an area of future interest, as PCP signaling in the eye is fundamentally

different from that in the wing. It also remains unclear whether such a PCP/Notch interaction occurs during vertebrate development.

Vertebrate gastrulation

The first vertebrate process in which Dishevelled was found to govern planar cell polarity was convergent extension, a morphogenetic tissue movement involving simultaneous lengthening and narrowing of a tissue (Fig. 4C). This process involves the planar polarization of a sheet of cells, where polarity is manifested by stable lamellipodia that form specifically on the mediolateral faces of the cells, but not on anteroposterior faces (Fig. 4C,D,F). These mediolaterally oriented lamellipodia exert tension on neighboring cells, resulting in cell interdigitation (Fig. 4C,D,F) (Keller, 2002; Wallingford et al., 2002).

Dishevelled was implicated in convergent extension (Sokol, 1996), and later studies revealed that Dishevelled functioned in a PCP-like pathway to govern convergent extension in both frogs and fish (Heisenberg et al., 2000; Tada and Smith, 2000; Wallingford et al., 2000). Time-lapse imaging of cells revealed that Dishevelled has a conserved role in controlling cell polarity: disrupting XDSH function randomizes the normally polarized lamellipodial protrusions that drive convergent extension (Wallingford et al., 2000). As is the case for actin-rich prehairs in the fly wing, XDSH accumulates in the actin-rich lamellipodia of cells undergoing convergent extension (Kinoshita et al., 2003).

The importance of convergent extension for gastrulation is unlikely to be limited to fish and frogs (Solnica-Krezel, 2005). Indeed, the disruption of Dishevelled function inhibits convergent extension in the ascidian, a non-vertebrate chordate (Keys et al., 2002). Likewise, the importance of Dishevelled function during gastrulation is not limited to convergent extension. Studies indicate that Dishevelled signaling via the PCP cascade may govern several other cell behaviors during *Xenopus* and zebrafish morphogenesis (e.g. Ewald et al., 2004; Marsden and DeSimone, 2001; Matsui et al., 2005). Dishevelled may also signal via the WNT/Ca^{2+} pathway to govern additional aspects of amphibian gastrulation (Winklbauer et al., 2001). Future studies should aim to find the unifying features of the cellular events that are governed by this protein.

Neural tube closure

Convergent extension not only drives gastrulation, but also occurs during neural tube closure in vertebrate animals, including amphibians, chick and mice (Jacobson and Gordon, 1976; Keller et al., 1992; Lawson et al., 2001; Sulik et al., 1994; Van Straaten et al., 1996). Dishevelled is essential for the convergent extension of neural tissue (Wallingford and Harland, 2001). Moreover, disrupting Dishevelled function in *Xenopus* has demonstrated that midline convergent extension is a critical aspect of neural tube closure; it narrows the distance between the forming neural folds and facilitates neural fold apposition and fusion (Wallingford and Harland, 2002). This mechanism is conserved across vertebrates, as disrupting mouse *dvl1* and *dvl2* genes also causes neural tube closure defects (Hamblet et al., 2002). Importantly, the phenotype of DVL mutant mice is reminiscent of a severe human neural tube closure defect called craniorachischisis (Kirillova et al., 2000; Saraga-Babic et al., 1993).

Directed migration of individual cells

Since the initial finding that a vertebrate cognate of the PCP cascade governs cell polarity in vertebrates, many PCP genes identified in *Drosophila* have been examined in vertebrates and have been found to influence convergent extension (Mlodzik, 2002; Wallingford et al., 2002). Despite the similarities, there is a crucial difference in the functioning of the PCP cascade in *Drosophila* and in vertebrate convergent extension. In flies, cross-talk between neighboring cells via PCP signaling components allows feedback amplification that reinforces the polarity decision (Fig. 4A,B) (Tree et al., 2002). During convergent extension, the vertebrate PCP pathway coordinates movement in a population of cells that are constantly changing neighbors; therefore, no long-term reinforcement of polarity from a particular neighboring cell is possible (Fig. 4C,D).

The highly dynamic nature of vertebrate PCP signaling is highlighted by the finding that a loss of Dishevelled function disrupts not only cell polarity during convergent extension but also lamellipodial stability (Wallingford et al., 2000). Indeed, several recent studies demonstrate that Dishevelled controls dynamic cell protrusions not only during polarized cell movements of large tissue sheets, but also during directed migration of individual cells.

Foremost among these is the finding that Dishevelled-dependent PCP signaling governs the directed migration, but not the specification, of *Xenopus* neural crest cells (De Calisto et al., 2005). Additional studies suggest that such a function for Dishevelled is widely used. For example, Dishevelled is essential for the directed migration of CHO cells during wound healing (Endo et al., 2005), and PCP signaling (and thus very likely Dishevelled) governs the migration of cardiomyocytes during the development of the outflow tracts of the heart (Phillips et al., 2005) (see also Hamblet et al., 2002).

Importantly, time-lapse studies in a variety of cell types hint at the mechanism by which Dishevelled contributes to directed cell migration. During convergent extension, cells lacking Dishevelled function are not only de-polarized, but their lamellipodial protrusions become highly unstable (Wallingford et al., 2000). Likewise, Dishevelled is required to stabilize leading-edge lamellipodia in migrating neural crest cells (De Calisto et al., 2005). A similar effect was recently reported in post-embryonic cells; disruption of DVL2 function with siRNA in bovine arterial endothelial cells caused lamellipodia to undergo rapid extension and retraction (Wechezak and Coan, 2005). Finally, a strong correlation between Dishevelled function and the stability of cell protrusions has been observed in cultured Saos-2 cells (a human osteogenic sarcoma-derived cell line) (Wiggan and Hamel, 2002).

This role is not limited to vertebrates. In the *Drosophila* ovary, Dishevelled is required for proper cell migration during ovariolar morphogenesis (Cohen et al., 2002). In this case, WNT, Frizzled and Dishevelled are involved, but other components of the PCP or canonical WNT pathways are not. As in vertebrates, this defect in cell migration does not result from defective polarity, but rather from the inability of cells to move productively (Cohen et al., 2002). This phenotype in the *Drosophila* ovary correlates with a failure to accumulate focal adhesion kinase (Cohen et al., 2002), raising the possibility that cross-talk occurs between Dishevelled and the adhesion machinery of a cell. Notably, adhesion to fibronectin has been

found to cause Dishevelled to translocate to the cell membrane (Marsden and DeSimone, 2001), and Dishevelled also associates with Ephrins and Eph receptors (Tanaka et al., 2003).

So far, the studies of Dishevelled function in individual cell migration demonstrate a role in stabilizing protrusions. Future studies should determine whether or not Dishevelled also plays a role in orienting the leading edge of migratory cells. It will also be of interest to determine whether other PCP genes share this functionality with Dishevelled, and to elucidate the ways in which this signaling cascade has changed to accommodate the dynamic nature of motile cells. In light of these findings, it is interesting to note that, from studies of planar polarization of the insect denticle following wounding, Nubler-Jung and colleagues presciently commented many years ago that 'cell migration and denticle formation may thus share similar orienting mechanisms' (Nubler-Jung et al., 1987). It will be important now to learn just how similar these mechanisms really are.

Polarized cell division

Polarized cell divisions contribute to morphogenesis in a wide variety of systems, and Dishevelled controls division polarity in several cell types. What is peculiar is that Dishevelled may direct oriented cell divisions through multiple, highly divergent mechanisms.

Dishevelled was first implicated in governing the orientation of cytokinesis in *Drosophila* sensory organ precursor cells. These cells divide in the plane of the epithelium, oriented along the anteroposterior axis, but this polarity is randomized when Dishevelled is disrupted (Gho and Schweisguth, 1998). Because the PCP-specific *dsh*^{1} allele or mutations in other PCP genes (e.g. *flamingo*) disrupt this polarity, it is likely that Dishevelled signals via a PCP cascade to control these polarized cell divisions (Bellaiche et al., 2001; Gho and Schweisguth, 1998; Lu et al., 1999).

This aspect of Dishevelled function is conserved in vertebrates; a recent study found that dominant-negative Dishevelled constructs disrupt polarized cell divisions in the gastrulating zebrafish. Here, again, Dishevelled acts in a PCP-like signaling cascade, as manipulation of the PCP effector Strabismus/Vangl2 also randomizes these normally polarized divisions (Gong et al., 2004).

Dishevelled also controls a variety of polarized cell divisions in the developing *Caenorhabditis elegans* embryo. Curiously, the orientation of these mitoses requires Frizzled, Dishevelled and GSK-3, but not other components of the WNT or PCP pathways (Chang et al., 2005; Schlesinger et al., 1999; Walston et al., 2004).

Cell division orientation is coordinated by microtubules in the mitotic spindles and asters (e.g. Bellaiche et al., 2001; Kaltschmidt et al., 2000). It is therefore intriguing that Dishevelled can regulate microtubule stability via GSK3 (Ciani et al., 2004; Krylova et al., 2000). Indeed, GSK-3 has been shown to be an essential mediator of normal mitotic spindle dynamics (Wakefield et al., 2003). This aspect of Dishevelled function is only just emerging, but should be of particular interest, as it may represent yet another branch of Dishevelled signaling.

Cardiac development

Following the establishment of the body axis, Dishevelled signaling is re-used during organogenesis. For example, several studies implicate WNT signaling in the control of cardiac development (Olson and Schneider, 2003), and all three major branches of Dishevelled signaling appear to be involved.

The canonical WNT/β-catenin pathway can negatively regulate cardiogenesis at an early stage by suppressing the differentiation of the cardiomyocyte precursors derived from the mesoderm (Lickert et al., 2002; Tzahor and Lassar, 2001). By contrast, a DVL2-dependent WNT signal also triggers a cascade of signals through β-catenin, PITX2 and Cyclin D2 to promote cell proliferation within the cardiac outflow tract (Kioussi et al., 2002). Interestingly, the outflow tract defects in *Pitx2* knockout mice resemble those in *Dvl2* knockout mice (Hamblet et al., 2002).

This last result suggests that the only role for DVL2 in heart development may be to signal through β-catenin and activate gene expression. However, it is likely that DVL2 also transduces crucial PCP signals during outflow tract development, as the PCP effector Strabismus/Vangl2 is required for the proper migration of cardiomyocytes during the development of the outflow tracts of the heart (Phillips et al., 2005).

Finally, studies have shown that, in *Xenopus*, WNT11 controls early differentiation and morphogenesis in the heart (Pandur et al., 2002). In this setting, WNT11 acts through the DEP and PDZ domains of Dishevelled to activate PKC and then JNK (Garriock et al., 2005; Pandur et al., 2002), suggesting that the WNT/Ca^{2+} pathway may be involved. Additional studies will be necessary to pare out which pathways are influencing which aspects of cardiac development.

Vertebrate neuronal development and social behavior

Mice lacking DVL1 exhibit defects in social behavior, such as in sleeping behavior, nest building and whisker trimming (Lijam et al., 1997; Long et al., 2004), although the mechanism underlying this phenotype remains elusive. The canonical WNT pathway plays a role in the development and patterning of the brain. In fact, it has been reported that Dishevelled can convert naive ectoderm into neural tissue (Sokol et al., 1995), an effect that is likely to be mediated by the ability of canonical WNT signaling to repress BMP transcription in the ectoderm (Baker et al., 1999). A more established role for canonical WNT signaling is in neural patterning, where WNT signals posteriorize neural ectoderm, generating anteroposterior pattern in the central nervous system (McGrew et al., 1997; McGrew et al., 1995). Indeed, Dishevelled has the ability to mediate this transformation (Itoh and Sokol, 1997).

Dishevelled also appears to be important for later events in neural development, governing the morphology, differentiation and function of neurons in vertebrates (e.g. Fan et al., 2004; Kishida et al., 2004; Luo et al., 2002; Schulte et al., 2005). In particular, two studies highlight Dishevelled's ability to wear more than one hat. First, it has been shown that the atypical receptor tyrosine kinase RYK can act as a WNT co-receptor (Lu et al., 2004a). RYK forms a complex with Frizzled and WNT, and also with Dishevelled, to transduce a canonical WNT signal that is important for normal neurite outgrowth from dorsal root ganglia, and for the guidance of axons in the craniofacial motor nerves and the ophthalmic nerves in the mouse (Lu et al., 2004a).

In the second study, DVL1 was found to control the complexity of dendritic arbors in hippocampal neurons (Rosso et al., 2005). In this capacity, DVL1 signals via a PCP cascade, collaborating with WNT7b, RAC and JNK. Strikingly, in these neurons, DVL1 was found to co-localize with microtubules in the axons and with actin in cellular protrusions at the leading edge of extending neurons (Rosso et al., 2005). This latter association may be related to the effects of Dishevelled on lamellipodial stability (e.g. De Calisto et al., 2005; Wallingford et al., 2000; Wechezak and Coan, 2005), whereas the former may be related to the ability of Dishevelled to regulate microtubule stability (e.g. Ciani et al., 2004; Krylova et al., 2000). Further studies of the function of Dishevelled in neuronal development and function should be both illuminating and important.

Conclusion

Dishevelled signaling is an important regulator of a wide variety of signaling pathways and thus governs many important developmental processes. Some of these roles are very highly conserved across species, whereas others are not. In some cases, similar biological processes appear to require Dishevelled, but they need it to signal along divergent pathways. As we move forward, care should be taken. We should endeavor to find unifying features that will help us to understand this protein. But we should also be ready for the surprises that may be lurking in the next experiment.

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